DEVELOPMENT OF THE ANTERIOR PART OF THE MANDIBLE AND THE MANDIBULAR DENTITION IN TWO SPECIES OF CICHLIDAE (TELEOSTEI)

by

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ABSTRACT. - This paper presents a description of the early post-embryonic development of the anterior part of the mandible (Meckel's cartilage, mentomeckelian and dentary bones) and of the dentition in two species of cichlids, the substrate brooder *Hemichromis bimaculatus* and the mouth-brooder *Astatotilapia burtoni*, based on serial semithin sections and reconstructions. In both species, the first bone (dentary) appears in a parachondral position, separated from the perichondral bone Anlage (mentomeckelium). Its deposition coincides with mouth opening and appearance of the first tooth germs. The first dentition in both species is basically symmetrical and teeth are most likely initiated in an alternate manner. The tooth germs become attached to the supporting bone by means of a collar of attachment bone, which is argued to develop in connection with the dental unit but to need the stimulus of adjacent bone. Some germs however remain unsupported for at least the period which has been examined and act possibly as replacement teeth. Erosion of Meckel's cartilage is initiated only opposite such unsupported but well-developed tooth germs. Afterwards, separate erosion cavities merge to create a large medullary cavity. The possible role of the teeth in the initiation of cartilage resorption is discussed.

RÉSUMÉ. - Le développement postembryonnaire précoce de la partie antérieure de la mandibule (cartilage de Meckel, os mentomeckelien et dentaire) et de la dentition sont décrits, à partir de coupes semi-fines sériées et à l'aide de reconstructions, chez deux Cichlidae, *Hemichromis bimaculatus* et *Astatotilapia burtoni*. Chez ces deux espèces, le premier os (le dentaire) apparaît dans une position parachondrale, à distance de l'ébauche de l'os périchondral (l'os mentomeckelien). Sa formation coïncide avec l'ouverture de la bouche et l'apparition des premiers bourgeons dentaires. La première dentition est en général symétrique chez les deux espèces et les dents se mettent en place très probablement d'une façon alternée. Les bourgeons dentaires s'attachent sur l'os de support par une couronne d'os d'attache. Cet os est supposé se développer en relation avec l'unité dentaire mais cela nécessite le stimulus d'un os adjacent. Certains bourgeons restent dépourvus d'os d'attache, pendant au moins la période étudiée, et sont probablement des dents de remplacement. Le cartilage de Meckel est résorbé uniquement en face de tels bourgeons dentaires bien développés. Plus tard, les cavités d'érosion se rejoignent pour former une grande cavité médullaire. Le rôle que pourraient jouer les dents comme initiateurs de la résorption du cartilage est discuté.

Key-words. - Cichlidae, *Astatotilapia burtoni*, *Hemichromis bimaculatus*, Mandible, Meckel's cartilage, Dentary, Teeth, Ossification, Resorption.

Throughout the vertebrates, the mandible is supported by a cartilaginous rod, Meckel's cartilage, which is invested with bones during the early life history of the animal (embryonic, or as in certain teleosts, post-embryonic). It is well-established that, during ontogeny, Meckel's cartilage undergoes destructive changes varying from restricted resorption as in many teleosts, to profound transformation as in mammals (cf. Starck, 1979). In an attempt to find more details on the

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ontogenetic changes taking place in Meckel's cartilage in larval and juvenile fish, we were surprised by the scarcity of data available on the mechanism and timing of breakdown of Meckel's cartilage and hence of the normal development of the lower jaw in teleosts.

In recent years, we have been investigating the development of the lower jaw in cichlids, a successful family of perciform fishes. We have been led to this study by the peculiar interactions that seem to occur between tooth development, cartilage resorption and bone formation in the pharyngeal jaws of these fishes (the latter being modified hind branchial arches and extremely important tools in food processing) (cf. Huysseune, 1983, 1989). We have been particularly concerned with the question of whether teeth would also play a role as an inducer of cartilage erosion during the development of the mandible. Our broader aim is to find out whether the assumed important role of teeth in the normal morphogenesis of jaw elements is more widespread than hitherto assumed.

In this first of a series of three papers, we aim at a precise description of the early post-embryonic development of the dentition and of its anatomical relationship with the cartilage and bone in the anterior, dentigerous part of the mandible. The paper presents data on two cichlid species, *Astatotilapia burtoni*, a mouth-brooding species, and *Hemichromis bimaculatus*, a substrate brooder.

MATERIAL AND METHODS

Both *Astatotilapia burtoni* and *Hemichromis bimaculatus* fry were reared in the lab in small tanks, at 27 and 24°C respectively. In the mouth-brooder, larvae were removed from the mother's mouth and raised separately. Stages were collected according to the staging system of Otten (1982) in *A. burtoni* (i.e. at approximately daily intervals) and at daily intervals in *H. bimaculatus*. *A. burtoni* fry were fed minced Tetramin whereas *H. bimaculatus* fry were fed *Artemia* nauplii.

In the present study, stages of *H. bimaculatus* are expressed in number of days after hatching. Stage numbers of *A. burtoni* correspond to the stage numbers given by Otten (1982) for *Astatotilapia elegans*. Because hatching takes place in the mother's mouth in *A. burtoni*, the approximate time after hatching for this species is given between brackets.

The larvae were fixed in a mixture of 1.5% paraformaldehyde and 1.5% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.4) for 2 hours at room temperature. The specimens were then decalcified for 5 days at 4°C in 0.1M EDTA (disodium salt) added to the fixative and changed daily. The samples were subsequently rinsed in 0.1M sodium cacodylate buffer containing 10% sucrose and postfixed for 2 hours at room temperature in 1% OsO_4 in 0.1M sodium cacodylate buffer containing 8% sucrose. After rinsing in the same buffer they were dehydrated in a graded series of ethanol, impregnated in a mixture of propylene oxide and epon and embedded in epon. Serial semithin (2 μm) sections were cut with glass knives and stained with toluidine blue. These served to prepare both hand-made (Verraes, 1974) and computer-aided (Vanden Berghe *et al.*, 1986) reconstructions.

RESULTS

Structure of the cichlid mandible

The mandible of adult cichlids is composed of the remains of Meckel's cartilage and of six bones: two dermal bones, the dentary and angular bones, and four peri- and/or endochondral bones, the mentomeckelian (or mentomandibular), the coronomeckelian, the articular and retroarticular bones (cf. Daget, 1964; Ismail, 1979). Fig. 1 shows a computer-aided reconstruction of the right mandible of a specimen of *A. burtoni* at stage 10 (about 6.5 days after hatching). At this stage, Meckel's cartilage is still intact (apart from a small erosion cavity on the ventral side, not visible on this lateral view). It is surrounded by the two dermal bones and three out of the four perichondral bones which make up the adult mandible. The mentomeckelian bone, a perichondral ossification around the anterior part of each ramus, is continuous with the anterior portion of the dentary. The articular bone ossifies in two centers where the angular touches the perichondrium. The retroarticular bone is still a small perichondral ossification at the caudal end of Meckel's cartilage, beyond the articulation with the palatoquadrate; it has no connections with the angular. Only the coronomeckelian bone, a partly perichondral and partly intratendinous ossification in front of the lower jaw articulation, is still lacking. As the neuromasts still lie superficially, there is no splenial component yet so that the two dermal bones at this stage should be correctly called os dento-mentomeckelium and os angulo-articulare. The dentigerous area is confined to the anterior portion of the dentary. In due order, I will describe the appearance of Meckel's cartilage and the dentary, the development of the dentition and finally the relationship of the teeth to the underlying skeletal structures.

Development of cartilage and bone (Fig. 2)

In *Hemichromis bimaculatus*, one day after hatching, the buccal cavity is still closed (there is no aperture to the exterior). Meckel's cartilage is in a late blastema or early primordium stage. There is no trace yet of any dermal or perichondral ossification nor of any tooth germs. Rostrally both rami develop in one continuous condensation.

The first dermal bone Anlage in *H. bimaculatus* appears along with the first pair of tooth germs at two days after hatching. Specimens of this age which have their buccal cavity open to the exterior via an aperture show one tooth germ on each hemimandible along with the first bone matrix of the dentary caudal to this germ and well separated from it (Fig. 2a). The bone develops in a parachondral position on the lateroventral side of the front part of each ramus. However, in

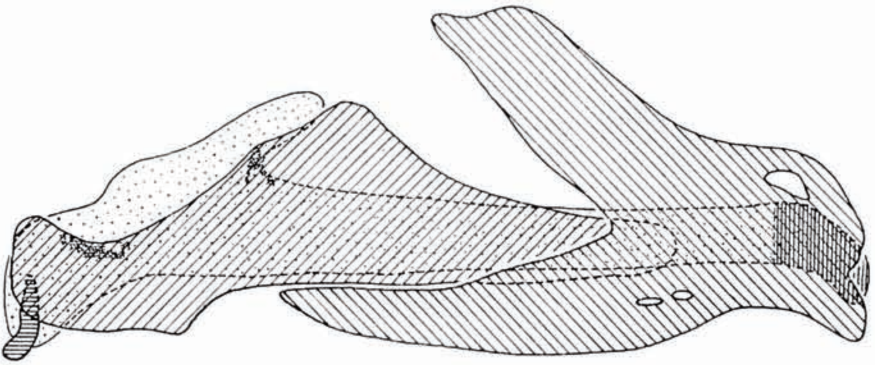
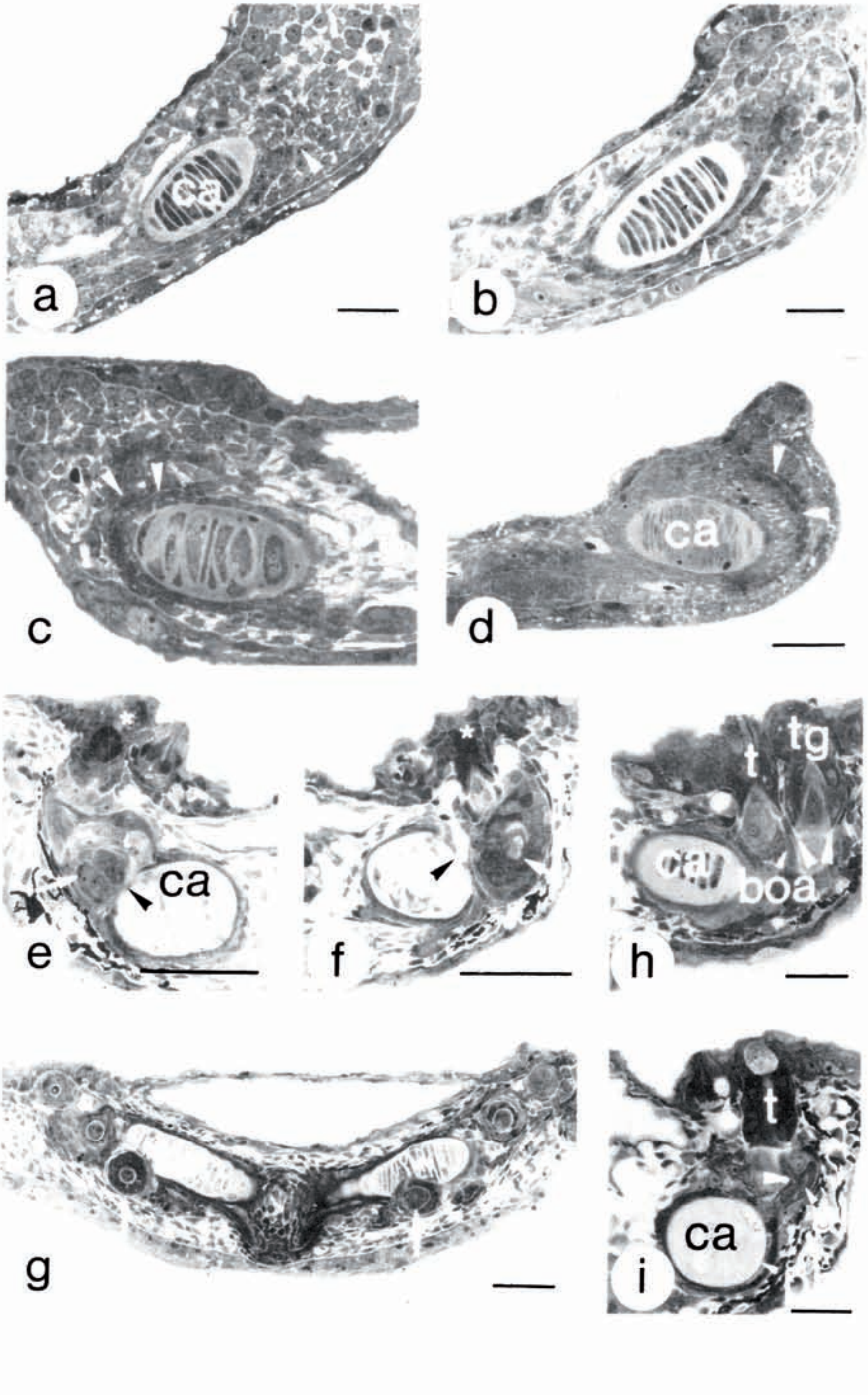


Fig. 1. - Graphical reconstruction (lateral view) of Meckel's cartilage and surrounding bones in stage 10 *Astatotilapia burtoni*. 1: Meckel's cartilage; 2: os dento-mentomeckelium; 3: os angulo-articulare; 4: os mentomeckelium; 5: os articulare; 6: os retro-articulare; R: rostral; D: dorsal. The mandible is 670 μm long.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specimens of two days where the mouth is not yet open, there are no tooth germs nor is there evidence of imminent ossification.

At three days, the least developed specimens still have only one tooth germ on either side. Yet, the Anlage of the dentary bone shows connections to the surface of the cartilage (Fig. 2b). Although perichondral bone around the rostral part of each ramus is barely visible at the light microscopical level at this age, signs of imminent perichondral ossification (such as an altered stainability of the cartilage surface) occur about simultaneously with the first appearance of the Anlage of the dentary.

Well developed specimens of three days after hatching already possess three tooth germs on either side. A second ossification center has appeared below the caudalmost tooth germ. This Anlage is connected to the perichondral bone, which has become more distinct by now (Fig. 2c). This second ossification center is also continuous, more caudally, with the Anlage of the dentary. From now on, the dentary quickly develops into a complex of perichondral and parachondral bone lamellae in which separate ossification centers can no longer be distinguished.

Astatotilapia burtoni having a longer embryonic period, chondrogenesis is ahead at one day after hatching or even less (stage 3 of Otten, 1982) as compared to *H. bimaculatus*. Yet, the mouth is still closed by a thin epithelial sheet and there is no dermal bone nor are there tooth germs. At stage 4 (about 1 day after hatching), the mouth is open and at the same time, the first dermal bone Anlage becomes visible, along with but caudal to the first tooth germ (Fig. 2d). Slightly different staining of the cartilage periphery announces imminent perichondral ossification from stage 3 onwards. Perichondral bone is well established from stage 5 (about 1.5 days post-hatching) onwards.

A few days after the cytodifferentiation of Meckel's cartilage, the first erosion cavities appear. Resorption of the cartilage starts at a slightly different age and at different standard lengths in both species: between 6.6 and 6.9 mm in *A. burtoni* (about 4.5 to 5.5 days after hatching) and between 4.5 and 5.0 mm in *H. bimaculatus* (i.e. at about 11 days after hatching). In both species, erosion is

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Fig. 2. - Transverse sections through the anterior part of the mandible in *Hemichromis bimaculatus (Hb)* (a-c, e-f and h-i) and *Astatotilapia burtoni (Ab)* (d and g). al: apolamella; boa: bone of attachment; ca: Meckel's cartilage; d: dentary bone; pb: perichondral bone; t: tooth; tg: tooth germ.

a: *Hb*. Well-developed specimen of 2 days post-hatching. Anlage of the dentary in a parachondral position (arrowhead) caudal to the only tooth germ present at this stage. Scale bar = 20 μm.

b: *Hb*. Specimen of 3 days. The Anlage of the dentary shows a matrix connection to the cartilage surface (arrowhead). Scale bar = 20 μm.

c: *Hb*. Advanced specimen of 3 days. Another ossification center (arrowheads) has appeared below the caudalmost tooth germ (not visible on this section). Scale bar = 20 μm.

d: *Ab*. Stage 4. Anlage of the dentary in a parachondral position (arrowheads). The only tooth germ is located more anteriorly. Scale bar = 40 μm.

e, f: *Hb*. 11 days. Right (e) and left (f) mandible in the same section. The pictures show the onset of erosion (arrowheads) opposite the pair of tooth germs (arrows) adjacent to those in position 6. The tooth germs involved in resorption are located below bone-supported teeth (asterisks). Scale bar = 40 μm.

g: *Ab*. Stage 9. Section showing the two rami of Meckel's cartilage and resorption opposite the pair of tooth germs in position 3 (arrows). All the teeth have a rostro-caudal orientation so that they are hit transversely. Scale bar = 40 μm.

h: *Hb*. 9 days. Attachment bone (arrowheads) is being deposited below a tooth germ in contact with an adjacent collar of attachment bone. Scale bar = 20 μm.

i: *Hb*. 9 days. Tooth with its pedestal partly attached to the apolamella projecting from the perichondral bone. Note the isthmus between apolamella and attachment bone of the tooth (arrowhead). Scale bar = 20 μm.

confined to portions of the cartilage surrounded by perichondral bone. Several small erosion cavities merge to create a large medullary cavity in the front part of each ramus. The cartilage of the symphyseal area however persists for a longer while. The erosion front moves posteriorly without prior matrix calcification.

Development of the dentition

The dentition as well as the anterior portion of each hemimandible of early postembryonic stages of *Hemichromis bimaculatus* and *Astatotilapia burtoni* were reconstructed from serial sections (Figs 3 and 5 respectively). To simplify, the dentitions were redrawn schematically (Figs 4 and 6 respectively). To allow comparisons to be made between subsequent stages, tooth positions were numbered consecutively from the front to the back of the jaw. Position number 1 corresponds to the foremost position in the tooth row at 5 days post-hatching in *Hemichromis bimaculatus* or at stage 6 in *Astatotilapia burtoni*. Only in the latter species does a germ irregularly develop in front of this position.

Hemichromis bimaculatus. The dentition at 5, 7, 9 and 11 days after hatching is pictured in Figs 3 and 4. As said earlier, one pair of young germs is present at two days. At three days, well developed specimens possess three pairs of germs, the middle one of which is most developed. During further development, the most striking features can be summarized from the reconstructions and especially from the schemes derived from them as follows.

1) The pattern on the left and right ramus is symmetrical, that is, teeth occur in the same positions and their developmental stage is similar on either side. Supernumerary tooth germs in the 5 day stage are weakly developed.

2) There is no clearly decreasing level of tooth germ development towards the front or rear end of each tooth row. Young germs are located at the front and at the rear end of each tooth row as well as in between.

3) The first teeth to possess a bony support do not lie adjacent to one another. At 7 days, bone-supported teeth alternate with less developed tooth germs.

4) The schemes from subsequent stages can readily be superimposed which allows one to compare teeth in similar positions in subsequent stages and so to trace their developmental history. In this way, it is possible to trace back one pair of germs (position 3) which is surrounded by more developed teeth, but which remains itself unsupported by bone throughout the six day period examined. Similarly, another pair of tooth germs developing later along the labial side of the row (next to position 6) does not become supported by bone either. The most striking feature shared by these two pairs is their location exactly opposite the first erosion cavities in the cartilage at 11 days (Figs 2e,f; indicated by arrows on Fig. 4D).

Astatotilapia burtoni. The situation is slightly different. A first pair of tooth germs appears at stage 4. At stage 5, three germs are present on either side. Of the three, the middle one is the most developed, but none of them is supported by bone yet. The dentition of stages 6 to 9 is pictured in Figs 5 and 6. The most striking features are:

1) Although the pattern on the left and right ramus is fairly symmetrical as to number, position and developmental stage of the tooth germs, there is a little more variation than in *H. bimaculatus*. Supernumerary tooth germs are present on one side at stages 6, 8 and 9, but these are often weakly developed. Similarly, a germ may be slightly more developed than its contralateral homologue, e.g. at stages 7 and 8.

2) Young germs may be found on the rostral side of the tooth row, on the caudal side or in between.

3) As in *H. bimaculatus*, bone-supported teeth are found in alternate positions.

4) Again, the dentitions can be superimposed, from which data on the developmental history of particular germs can be derived. So, as in *H. bimaculatus*,

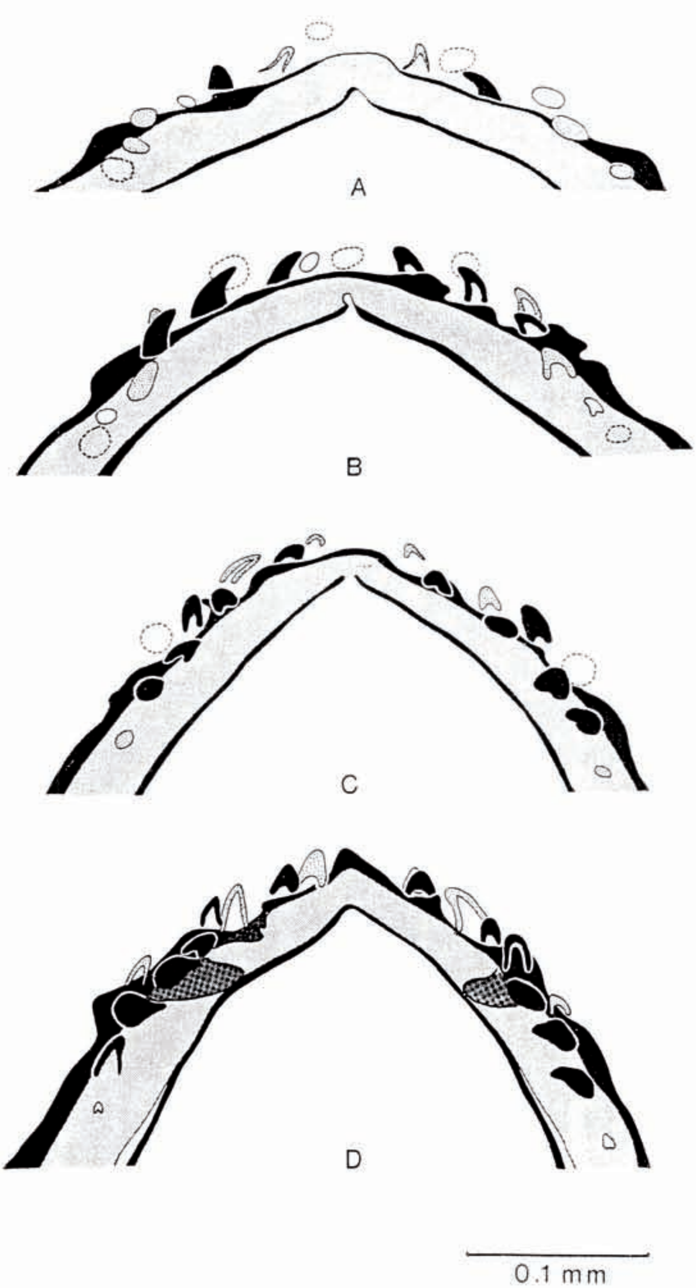

Fig. 3. - Reconstruction from serial sections (dorsal view) of the dentition and the rostral part of Meckel's cartilage in four early developmental stages of *Hemichromis bimaculatus:* 5, 7, 9 and 11 days post-hatching (A, B, C and D respectively). Young tooth germs up to bell stage (stages a and b, see Huysseune, 1983) are represented by dotted lines, tooth germs at a stage of early matrix deposition (stage c) are represented in white whereas well-developed mineralized germs (stage d) are dotted. Black teeth are those provided with a bony support. Bone is black; cartilage is stippled; erosion cavities are densely stippled.

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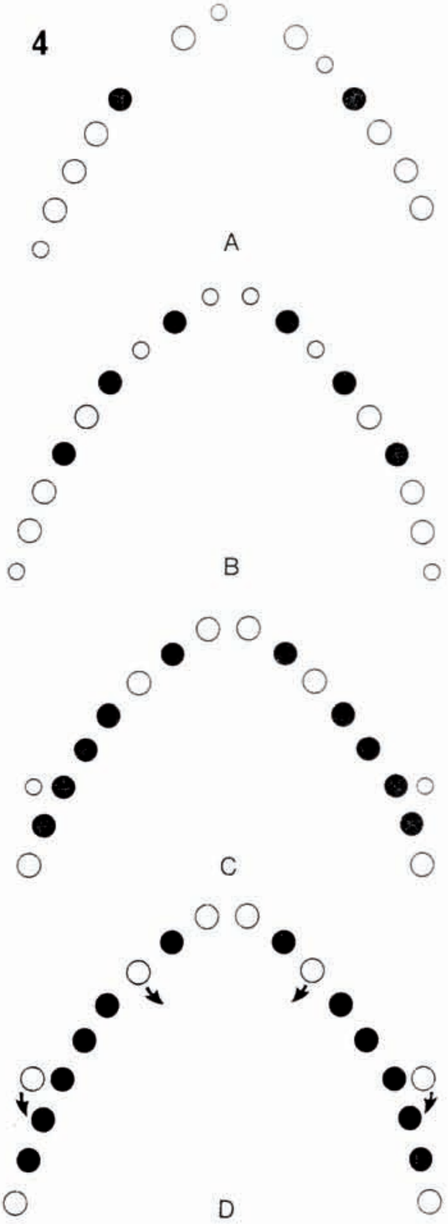

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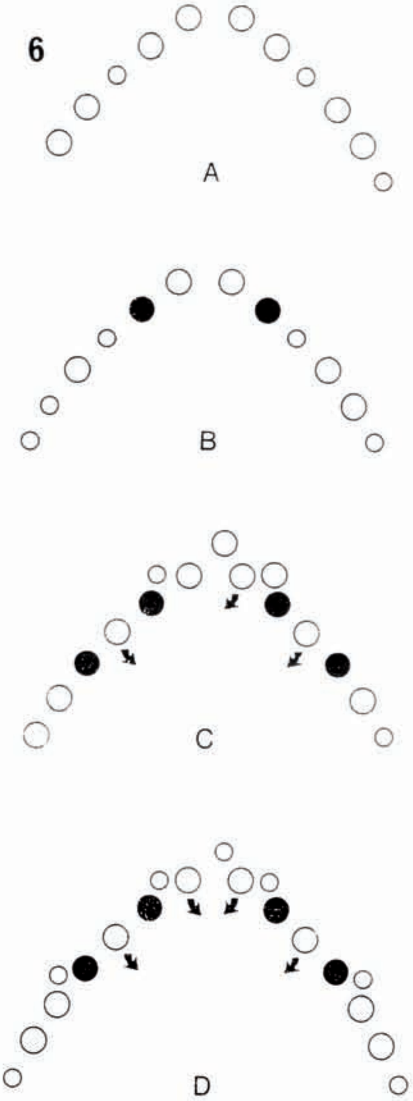

Fig. 4. - Schematic representation of the dentition in *Hemichromis bimaculatus* at 5, 7, 9 and 11 days post-hatching (A, B, C and D respectively), based on the reconstruction shown in Fig. 3 and on observations from sections. Germs in stages a and b are visualized by small circles, germs in stages c and d by larger circles, and teeth with a bony support by black dots. Arrows indicate those tooth germs that are found associated with cartilage resorption.

Fig. 6. - Schematic representation of the dentition in *Astatotilapia burtoni* at stages 6, 7, 8 and 9 (A, B, C and D respectively), based on the reconstruction shown in Fig. 5 and on observations from sections. Representation of tooth germs as in Fig. 4.

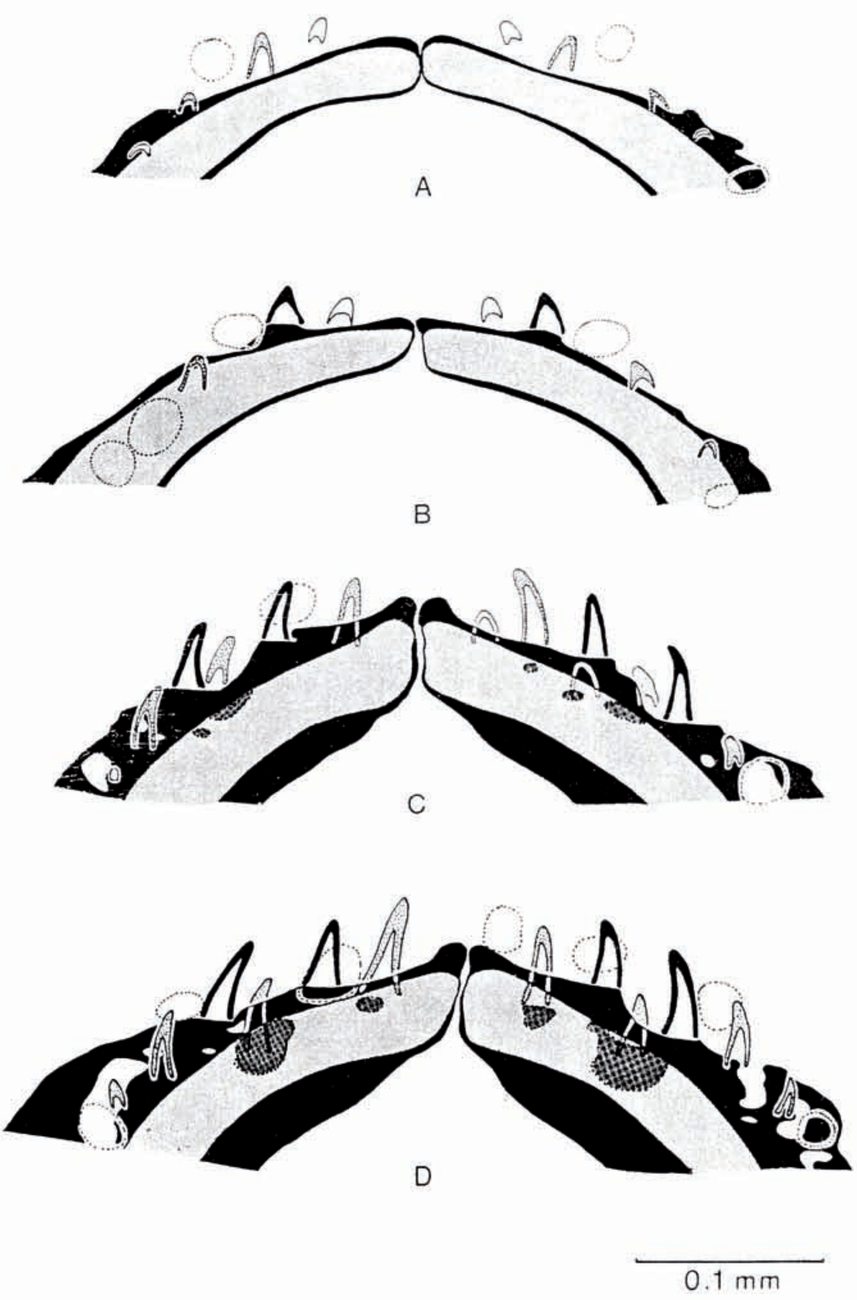

Fig. 5. - Reconstruction (dorsal view) of the dentition and the rostral part of Meckel's cartilage in four early developmental stages of *Astatotilapia burtoni*: stages 6, 7, 8 and 9 (A, B, C and D respectively). Representation of tooth germs as in Fig. 3. The bone is composed of a network of trabeculae; white areas in the bone represent spaces between these and are filled with connective tissue and vascular and nervous elements.

a pair of tooth germs (position 3) remains unsupported by bone during the period examined and lies itself between bone-supported teeth. Like in *H. bimaculatus*, its position matches the place of initial cartilage resorption starting at stage 8 (Fig. 2g). Cartilage resorption is also found opposite a pair of tooth germs rostral to the foremost bone-supported teeth (position 1). At stage 8 however, one germ of this pair lies close to the perichondral bone but the cartilage below does not yet suffer resorption. The two pairs of germs seemingly associated with resorption belong to the least developed germs at stage 6.

The orientation of the teeth with regard to the cartilage ramus slightly differs between the two species (compare Figs 2e, f, g and Figs 3, 5). In *Astatotilapia burtoni*, the teeth are inclined and their tip points rostrally. In *Hemichromis bimaculatus*, the teeth are oriented more or less perpendicularly to the long axis of each ramus (Figs 3, 5), except for those teeth that are associated with resorption. Indeed, these are oriented in a rostro-caudal direction, more or less parallel to each cartilage ramus.

Relationship between dentition and underlying skeletal structures

In the two species, bone-supported teeth have erupted or are about to erupt. Teeth become supported by bone once a so-called collar of attachment bone has developed below them (Fig. 2h). This consists of a collar of bone (a pedestal) separated from the tooth base by a weakly staining (presumably unmineralized) zone, as has been described for the pharyngeal jaws in *Astatotilapia elegans* (see Huysseune, 1983). Such collars are never seen to develop independently; there is always contact with other bone. In *Hemichromis bimaculatus*, the teeth in positions 2 and 4 have their collars fused directly to the perichondral bone. The pedestal of the tooth in position 5 is initiated adjacent to the pedestal of the tooth in position 4 (Fig. 2h) but fuses to perichondral bone in its rear portion. Teeth in more caudal positions are supported by a bony apolamella projecting from the perichondral bone but most likely deriving from the first parachondral ossification center reported earlier (Fig. 2i). Even here, there is at least a small collar of attachment bone connecting the tooth base to the apolamella. In *Astatotilapia burtoni*, teeth in positions 2 and 4 are supported by a collar of attachment bone which is connected to the perichondral bone by means of an osseous trabecula.

In *Hemichromis bimaculatus*, apart from the two pairs of tooth germs mentioned earlier, well-developed germs acquiring attachment bone before 11 days post-hatching may also be associated with resorption at one stage in their life-time (these tiny resorption cavities are not indicated on the figures). In these cases, the dental papilla closely adjoins the perichondral bone which is locally thin or is even pierced while showing superficial erosion of the cartilage matrix below. In stage 8 *Astatotilapia burtoni*, small erosion cavities are located below the dental papilla of three germs which also closely adjoin the perichondral bone (Fig. 5).

In both species, the tooth germs that are found in association with cartilage resorption may lie partially inside the corresponding erosion cavity (Figs 2e, f and g). In *A. burtoni*, the teeth penetrate Meckel's cartilage even from below (Fig. 2g). Fig. 7 shows a schematic drawing based on the reconstruction of the life-history of such a tooth germ. The dental organ elongates quickly thereby penetrating deeply into the mesenchyme. The dental papilla first grows around Meckel's cartilage which is surrounded in this area by the perichondral mentomeckelian bone (Fig. 7A). The tooth germ further elongates below the ossified cartilage, until at a particular moment perichondral bone and cartilage resorption is initiated opposite the dental papilla (Fig. 7B).

In *Astatotilapia burtoni*, the two separate erosion cavities present at stage 9 have merged by stage 12 (about 9 days after hatching), subdividing Meckel's cartilage into a small rostral nodule and the bulk of the ramus more caudally. At that time, the position of the rear teeth coincides with the posterior margin of the

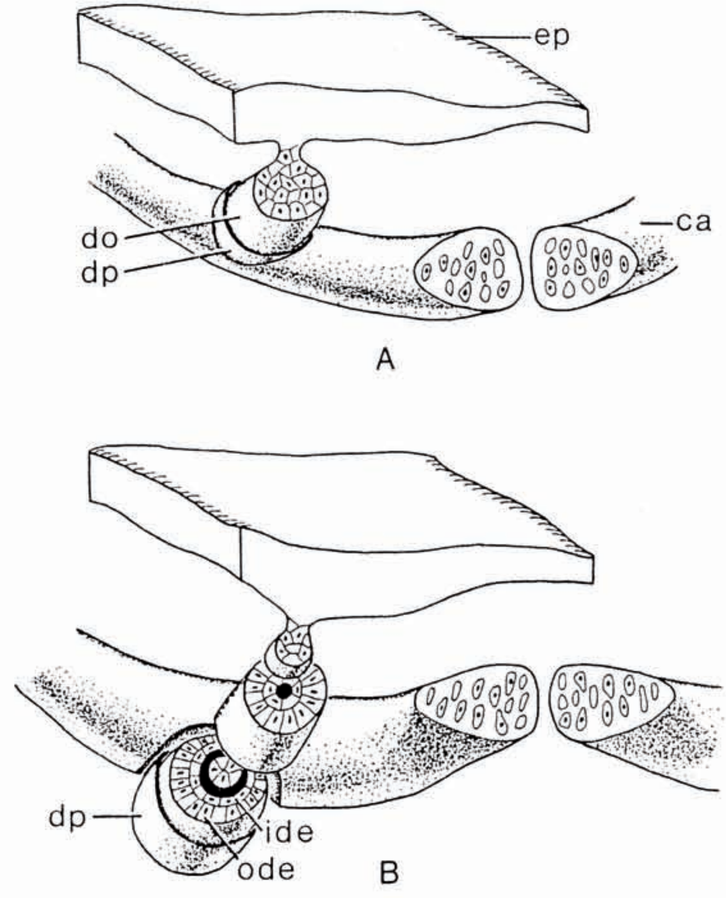

Fig. 7. - Schematic representation based on observations from sections showing how *A. burtoni* tooth germs elongate and penetrate Meckel's cartilage from below. Bone is not pictured. See text for details. ca: Meckel's cartilage; do: dental organ; dp: dental papilla; ep: buccal epithelium; ide: inner dental epithelium; ode: outer dental epithelium.

erosion cavity. In juveniles and adults however, the dentition stretches well beyond the eroded part of Meckel's cartilage.

In *Hemichromis bimaculatus*, erosion appears to be delayed. At 20 days post-hatching (5 mm SL), there are still only two minor cavities, each one invaded by an unsupported tooth. At 30 days (6 mm SL), there is a small rostral nodule of cartilage, separated from the bulk of each ramus by a large erosion cavity. Likewise, in this and older stages, the dentition stretches well beyond the posterior margin of the erosion cavity.

In neither of the two species has it been possible to trace with certainty attachment bone below teeth having invaded the erosion cavity. True endochondral bone, paving the erosion front of the cartilage, has neither been found.

DISCUSSION

Structure of the mandible

The structure of the mandible of *A. burtoni* conforms to the general teleost Bauplan (cf. Daget, 1964; Nelson, 1973) in having a central cartilaginous core surrounded by four perichondral and two dermal bones. Whereas Meckel's cartilage is homologous throughout the vertebrates (Hall, 1990), the bones that are associated with it certainly are not. The difficulty to distinguish the earliest deposits of perichondral bone in contrast to the ease with which the dermal bone Anlage can be recognized makes it hard to tell whether or not perichondral ossification precedes dermal ossification. To elucidate this, a detailed morphological study at the electron microscopical level is needed. The arguments put forth here in favour of the view that the perichondral bone should be regarded as a mentomeckelian, are: (1) the initial dermal (parachondral) ossification center develops separately from the cartilage; (2) during development, the perichondral bone stretches further backwards below the parachondral bone, so that in transverse sections, one can distinguish a perichondral bone collar separated by connective tissue from a parachondral bone collar (a situation also illustrated but not commented by Francillon, 1977 and also observed by Verraes, 1973); this observation led Verraes to doubt on the supposed absence of a perichondral mentomeckelian in *Salmo*. To consider that the parachondral dentary bone has first invaded the perichondrium in an anterior direction and then spread again posteriorly is unparsimonious; (3) except for the symphyseal area, the anterior portion of Meckel's cartilage is often subjected to an erosion process, which removes not only the cartilage but also its perichondral bone collar whence the difficulty to trace the latter in older post-embryonic stages; (4) the supposed phylogenetic loss of the mentomeckelian bone in teleosts is often based on data collected with inadequate techniques. As an example, many of the studies that deal with lower jaw structure in teleosts are based upon cleared specimens stained with alizarin, which are not suited for dealing with very tiny ossifications (recently Hanken and Hall (1988) warned for misinterpretations concerning the appearance of bones based on the use of whole mounts). Also, authors who have been claimed (e.g. by Francillon, 1977) to minimize the presence of a mentomeckelian do in fact regard the anterior ossification of Meckel's cartilage as a compound bone with a mentomeckelian and a dentary portion (e.g. Kindred, 1919 in *Amiurus*; Lekander, 1949 in *Phoxinus*; Jollie, 1975 in *Esox*). Based on these arguments, I conclude that the anterior ossification of Meckel's cartilage in the cichlids examined in this study is a compound bone resulting from the fusion of a (be it a relatively small) perichondral mentomeckelian and a dermal dentary, and is thus termed correctly 'dento-mentomeckelian'. In this sense, I agree with Patterson (1977) who rejects the possibility of invasion of Meckel's cartilage by the dentary. This conclusion does however not imply a denial of the fact that the mentomeckelian has lost importance during actinopterygian evolution (a conclusion also reached by Francillon, 1977). This is evident when comparing the limited ossification in teleosts with the independent ossification reported in primitive actinopterygians such as *Polypterus* and *Amia* (Pehrson, 1947; Nelson, 1973). I furthermore disagree with Haines (1937) who, by simply homologizing the dentary in a number of teleosts and by denying the importance that can be attached to the developmental origin of a teleostean bone, concludes that the mentomeckelian is probably absent in teleosts.

Development of bone

In both species the formation of the first dermal bone anteriorly coincides - at least under the light microscope - with the formation of the first tooth germs, and opening of the buccal cavity. The bone appears independently from the teeth and, in *Hemichromis*, only the second ossification center (which soon fuses to the first Anlage) seems to develop in relation with one of the tooth germs.

The attachment bone, which develops as a collar below each tooth and is separated from it by an uncalcified zone conforms to attachment mode type 2 of Fink (1981). There is much variation among teleosts in the way dentary teeth attach to the bone (see e.g. Levi, 1939a,b,c; Kerr, 1960; Shellis and Berkovitz, 1976; Kerebel *et al.*, 1979; Andreucci *et al.*, 1982; Motta, 1984; Howes and Sanford, 1987, to name some). The type of attachment may vary on one bone (e.g. in larval *Belone:* directly to the bone by means of upgrowths, or indirectly by means of an independently developing collar of attachment bone (Moy-Thomas, 1934); also: hinged versus ankylosed teeth in *Merluccius* (Levi, 1939c) and in *Esox* (Herold and Landino, 1970)).

The attachment bone always develops in continuity with other bone which makes it impossible to judge whether it derives developmentally from the dental unit or whether it is merely bone that comes to meet the dental base. The origin of the attachment bone, whether it is bone-derived (e.g. Moy-Thomas, 1934) or tooth-derived (e.g. Kerr, 1960; Shellis and Berkovitz, 1976) has been a matter of much controversy (cf. also Huysseune, 1983; Smith and Hall, 1990). Our present opinion is that the attachment bone is part of the tooth organ but needs a (permissive?) stimulus from adjacent bone to be deposited. Observations which support this view are: the presence sometimes of an isthmus between pedestal and supporting bone, the presence of what seems to be a cement line between both, and a seemingly different orientation of the fibres. On the upper pharyngeal jaws, collars of attachment bone form without any developmental connection with the endoskeleton (there is no dermal bone present) but need the presence of an adjacent collar to develop (the first collar being connected to the perichondral bone) (Huysseune, 1983), a situation which inevitably leads one to conclude that the attachment bone is derived from the tooth. There is however an alternative scenario which merits consideration. One can imagine the following evolutionary sequence: (1) the attachment bone forms as an outgrowth from the supporting bone; (2) as in (1) but calcification is limited to that part of the outgrowth adjoining the tooth base; (3) the connection with the bone is lost and only the mineralized zone close to the tooth base develops. Should such an evolutionary series exist, then the phylogenetic origin of the attachment bone has to be reconsidered.

Thus, in contrast to the upper pharyngeal jaws, the collars of attachment bone do not form the only ossification centers of the dentary. The collar of attachment bone is always fused *ab initio* with adjacent bone, be it perichondral bone, lamellae emanating from it or even other attachment bone. Thus the tooth-bearing bone is of mixed origin, much like on the lower pharyngeal jaws but unlike on the upper pharyngeal jaws, where it solely consists of fused collars of attachment bone (Huysseune, 1983). Earlier, we have suggested that differences in how the dentigerous bone is built up may be related to differences in shape and size of the original cartilaginous support. Upper and lower pharyngeal jaws have distinctively shaped cartilaginous supports (plate versus rod), whereas the lower pharyngeal jaw and lower jaw both have a rod-shaped cartilaginous support. The significance of this has to be further examined.

Development of the dentition and relations between teeth and skeleton

Although there has been an increased interest in the development and structure of teleost - even cichlid - fish teeth in recent years (Sasagawa, 1984, 1988; Sasagawa and Igarashi, 1985; Sasagawa and Ishiyama, 1988; Prostak and Skobe 1986a,b; Prostak *et al.*, 1989; Uehara and Miyoshi, 1987) there is still a considerable gap in our knowledge concerning the initiation of the dentitions in these fishes (exceptions to this are e.g. the studies of Berkovitz, 1977, 1978; Nakajima, 1987). The one-day interval between the stages examined in this study is too large to determine the exact order of tooth initiation. In both species, several new tooth germs may be added within 24 hours although at the time they are first seen, their developmental stage may differ. Assuming that the developmental stage reflects the order of tooth initiation, then the pattern of bone-supported (hence erupting) teeth indicates that teeth are initiated in an alternating manner in both species. At least in *Salmo gairdneri*, the order of eruption of the lower jaw teeth also reflects the order of initial tooth development (Berkovitz, 1977). In *Hemichromis*, the first tooth to appear would be in position 4, followed by positions 2 and 6. Then teeth would be initiated in the odd positions 7, 5, 3 and 1, followed again by even position 8. In *Astatotilapia*, the first tooth to appear would be in position 2, followed by 4, then, in order of appearance, positions 1, 5, 3, 6, 7. If sequential inductions occur, then the development of germs on alternate loci must be suppressed. In the largest upper pharyngeal jaw element of *Astatotilapia elegans*, a close relative of *A. burtoni*, teeth are initiated from medial to lateral and from caudal to rostral (Huysseune, 1983). That tooth initiation patterns do not necessarily follow alternating waves (said to be characteristic for lower vertebrates (cf. Lawson and Manly, 1973) or can differ among elements of the same (or in this case a closely related) species is confirmed by other authors. In *Salmo gairdneri*, teeth are initiated in an alternating manner from the front to the back in the maxillary and basihyal (Berkovitz, 1978) and dentary (Berkovitz, 1977) but sequentially in both directions on the premaxilla (Berkovitz, 1978). In piranhas, tooth initiation is also sequentially (Berkovitz and Shellis, 1978).

Several theories have been proposed to account for the generation of the tooth pattern in reptiles and mammals; Lumsden (1979) categorized them into either "gradient" theories (in which an external signal is assumed to produce the pattern, e.g. Edmund's (1960) Zahnreihen) or "cell-lineage" theories (in which the pattern is self-generated, e.g. Osborn's (1978) clone theory). The idea that innervation may play a (trophic or more directive) role in organizing the dentition has been suggested during the seventies (see Pearson, 1977; Kollar and Lumsden, 1979; Kollar, 1981) but has been refuted since. The truth is probably far more complicated and the inductive mechanisms determining tooth position and subsequent tooth form may lie in molecular patterning of the mesenchymal stroma and basal lamina (Kollar, 1981; 1983). Alternatively, a prepattern residing in the oral ectoderm may determine the sites of dental development in mammals (Lumsden, 1987).

In the present study, supernumerary teeth are usually weakly developed germs and it is assumed that the contralateral homologue will develop soon afterwards. The initial dentary dentition of both cichlid species is therefore considered to show basically a bilateral symmetry. Bilateral symmetry in the developing dentition is probably common but not the rule. Some piranhas e.g. show a close correlation in the stage of development between upper and lower dentitions on the same side but a phase difference between opposite sides of the jaws (Berkovitz, 1975; Shellis and Berkovitz, 1976), although other patterns are possible as well (Berkovitz and Shellis, 1978). Bilateral asymmetry is also found in pharyngeal tooth counts, e.g. in cyprinids (Evans and Deubler, 1955; Eastman and Underhill, 1973; Smith and Hocutt, 1981) but the authors regard this to be due to faulty replacement.

Both developmental series share a number of features regarding the relation between cartilage resorption and tooth development. First, the initial sites of cartilage resorption are located only opposite tooth germs and the cartilage invariably bears a perichondral bone where it suffers resorption. Second, resorption occurs opposite teeth which adjoin the cartilage laterally and even ventrally. Third, resorption only takes place opposite well-developed tooth germs but as yet unsupported by bone. Finally, the evidence available up to now suggests that the erosion site always adjoins the dental papilla.

We further speculate that only tooth germs in specific loci can be found in association with cartilage resorption. This speculation comes from the observation that tooth germs which will be involved in resorption, at least in *Hemichromis bimaculatus*, behave differently well before resorption is initiated. This leads me to believe that they are committed to "induce" resorption long before they reach the cartilage. Up to now, I cannot determine their precise fate, because (1) reconstructions of older, closely spaced stages are not available, (2) experimental techniques to enable the history of individual fish teeth to be traced are not yet available, and (3) due to the erosion process the cartilage boundary completely disappears in such a way that, once this erosion covers a certain distance, one cannot judge the exact position of a tooth with regard to the (former) cartilage boundary. The exclusive presence of unsupported teeth within recognizable parts of the eroded ramus suggests that their growth is retarded, or arrested. One possibility is that they represent replacement teeth (or teeth which take new positions in the dentition in a later phase, this has still to be sorted out). This could explain both their different orientation and their long phase as differentiated yet non-functional tooth. Whether the cartilage merely responds to the presence of such a differently behaving tooth germ (whatever its nature may be) through a physical or a chemical factor issuing from the tooth tissues, or whether the same signal determines this diverging tooth behaviour and induces resorption, is still enigmatic. On the other hand, the irregular occurrence of spatially and temporally limited resorption at sites where teeth closely adjoin the mandibular ramus may indicate that the mere nearby presence (pressure?) of the tooth is sufficient to initiate cartilage resorption. Clearly, ultrastructural and experimental data may provide clues in our understanding of the phenomenon.

The possible role of teeth in the initiation of cartilage resorption has been stressed for the pharyngeal jaws in cichlids (Huysseune, 1983, 1989). Quite interestingly, Francillon (1977) signals that the endochondral bone in the anterior part of Meckel's cartilage seems to have a relation with the attachment bone below the teeth. This may indicate that teeth may also be involved in the erosion process, but unfortunately the author does not give any evidence of this. One of the very few papers dealing with the possible role of teeth in the resorption of Meckel's cartilage in another vertebrate class is by Savostin-Asling (1974). She shows that the initial locus of resorption of the intermediate segment of Meckel's cartilage in the rat matches the area of the developing incisor tooth and quotes earlier papers, indicating that no such erosion system was observed in the "incisor-absent" strain of these rats. Mühlhauser (1983) describes an initial erosion center in Meckel's cartilage of the neonatal rat medial to the incisor, and a second 'molar' erosion center which is often overlooked because of its rapid incorporation into the growing erosion cavity.

The suspected role of teeth in the initiation of cartilage breakdown clearly awaits further exploration. Small osteoclasts have been identified at the resorption sites by transmission electron microscopy and have been described elsewhere (Sire *et al.*, 1990). Further ultrastructural observations are necessary to study the fine details of the tissues involved in the area prior to, during and after initial resorption.

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